Effects of peroral insulin and glucose on circulating insulin-like growth factor-I, its binding proteins and thyroid hormones in neonatal calves

Danijela Kirovski, M. Lazarević, Ivona Baričević-Jones, Olgica Nedić, Romana Masnikosa, Judith Anna Nikolić

Abstract

There is disagreement in the literature about the ability of neonatal calves to absorb perorally administered insulin. This study evaluated the absorption of a bolus of insulin administered alone or with an energy souce and its effects on the circulating insulin-like growth factor system and thyroid hormones in newborn Holstein-Friesian calves. Within 1 h of dosing, mean serum insulin and triiodothyronine (T3) concentrations had increased considerably, whether the insulin was applied alone (n = 4) or together with glucose (n = 4), accompanied by marked hypoglycemia. No significant changes were observed in control calves (n = 4) given the vehicle solution. Increased serum glucose and T3 concentrations with no change in insulinemia occurred in a 4th group of calves given glucose alone. At 32 h of age and after 3 meals of colostrum there were no differences in glycemia, insulinemia, or proteinemia among the 4 groups of calves examined. Mean serum insulin-like growth factor-I (IGF-I) tended to decrease over this period in the control group. The decrease was more pronounced in the insulin-treated group but absent in both groups that received glucose. These differences were associated with equivalent differences in abundance of the 40–45K IGF-binding protein-3 (IGFBP-3); however, lower molecular mass IGFBPs were not affected. The results show that a pharmacological peroral dose of insulin can lead to rapid systemic alterations in the IGF/IGFBP system in neonatal calves that can be modified by simultaneous administration of a small energy supply in the form of glucose.

Résumé

Il y a désaccord dans la littérature quant à la capacité des veaux nouveau-nés à absorber de l'insuline administrée per os. *Cette étude visait à évaluer l'absorption d'un bolus d'insuline administré seul ou avec une source d'énergie et ses effets sur le système des facteurs de croissance apparentés à l'insuline et les hormones thyroïdiennes chez des veaux Holstein-Friesian nouveau-nés. En moins d'une heure après l'administration, les concentrations sériques moyennes d'insuline et de triiodothyronine (T3) avaient augmenté considérablement, que l'insuline soit administrée seule (*n = 4*) ou avec du glucose (*n = 4*), et étaient accompagnées d'une hypoglycémie marquée. Aucun changement significatif n'a été observé chez des veaux témoins (*n = 4*) n'ayant reçu que le véhicule d'injection. Une augmentation des concentrations de glucose sérique et de T3 sans changement dans l'insulinémie a été notée dans un 4e groupe de veaux n'ayant reçu que du glucose. À 32 h d'âge et après 3 repas de colostrum il n'y avait pas de différence dans la glycémie, l'insulinémie et la protéinémie entre les 4 groupes de veaux examinés. La concentration sérique moyenne du facteur de croissance apparenté à l'insuline de type 1 (IGF-I) avait tendance à diminuer durant cette période dans le groupe témoin. La diminution était plus marquée parmi le groupe traité à l'insuline mais absente chez les deux groupes ayant reçu du glucose. Ces différences étaient associées avec des différences équivalentes dans l'abondance de la protéine liant l'IGF de type 3 de 40–45K (IGFBP-3); toutefois, les IGFBP de poids moléculaire plus faible n'étaient pas affectées. Les résultats montrent qu'une dose pharmacologique d'insuline administrée* per os *peut amener des altérations systémiques rapides dans le système IGF/IGFBP chez les veaux nouveau-nés qui peuvent être modifiées par une administration simultanée d'une petite quantité d'apport énergétique sous la forme de glucose.*

(Traduit par Docteur Serge Messier)

Introduction

After the stress of birth, newborn calves exhibit a specific hormonal status characterized by high circulating concentrations of corticosteroids and thyroid hormones (1,2). Many investigations on the development and interaction of the different hormonal axes have been made during the last 2 decades (3–6). In general, it has been concluded that the bioactive substances in colostrum primarily affect gastrointestinal development, while the systemic endocrine and metabolic state is mainly influenced by the nutritional components of colostrum and the time elapsing between birth and the 1st meal. The somatotrophic axis is functional in newborn calves and postnatal growth and development involves growth hormone, insulin-like growth factors (IGF) and insulin, together with their receptors, binding proteins, and proteases (7,8).

Most studies on the effects of insulin have involved the use of intravenous infusions of insulin under various glycemic clamp

Department of Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Belgrade, Belgrade Serbia (Kirovski, Lazarević); INEP — Institute for the Application of Nuclear Energy, University of Belgrade, Belgrade, Serbia (Baričević-Jones, Nedić, Masnikosa, Nikolić).

Address all correspondence and reprint requests to Dr. Danijela Kirovski; telephone: (+) 381-113-615-436/350; fax: (+) 381-112-685-936; e-mail: dani@vet.bg.ac.yu

Dr. Kirovski's current address is Faculty of Veterinary Medicine, Bulevar Oslobodjenja 18, 11000 Belgrade, Serbia.

This study formed part of the investigations included in the PhD thesis of Danijela Kirovski successfully defended at the Faculty of Veterinary Medicine in Belgrade in 2005.

conditions. However, many years ago Pierce et al (9) demonstrated that insulin administered orally to calves within 24 h after birth was absorbed in a biologically active form, leading to marked hypoglycemia; the effect could not be repeated by Grutter and Blum (10). Moreover, it was found that plasma glucose and insulin concentrations could be increased by long-R3-IGF-I only after parenteral administration, while oral doses had no effect (11).

Neonatal calves tend to develop hypoglycemia, which may be rectified by glucocorticoid administration (12). The increased plasma glucose concentrations, however, are not associated with stimulation of hepatic gluconeogenic enzyme activities, but with impaired peripheral insulin-dependent glucose utilization (13).

Our earlier work confirmed that the amount of colostrum consumed during the first 32 h of postnatal life in calves had marked effects on the serum IGF-I, cortisol, thyroid hormones, and total protein concentrations (2,14). The objective of the present study was to attempt to reproduce the results of Pierce et al (9) and to examine the effect of acute hyperinsulinemia on some elements of the IGF system and thyroid hormones; both intimately associated in the control of growth and metabolism in several mammalian species (15–19). A pharmacological dose of insulin was given perorally to calves immediately after birth alone or together with a physiological quantity of glucose 1 h before the first meal of colostrum. Responses were compared to those of calves receiving only vehicle or glucose.

Materials and methods

Calves

Sixteen calves (11 females and 5 males; Holstein-Friesian breed), born within a 2-wk period, were successively assigned to 1 of 4 groups in order: I, G, IG, C and placed in individual boxes in a byre where the temperature ranged from 15 to 20°C. The mean body weight (BW) at birth was 38.4 ± 3.6 kg with no significant difference between the males and females ($P > 0.05$). The animal-related component of the study was approved by the Ethical Committee of the Faculty of Veterinary Medicine, University of Belgrade in accordance with the National Regulation on Animal Welfare.

Solutions for treatment

All reagents were analytical grade (Merck, Darmstedt, Germany; Zorka, Sabac, Serbia). Stock solutions of crystalline porcine insulin (anhydrous potency 25 U/mg; Galenika, Zemun, Serbia) were prepared by dissolving in 0.01 M hydrogen chloride (HCl) until solution was obtained. These solutions (pH 1) were dispensed into small vials and stored at −20°C for a maximum of 2 d before use. Immediately before administration, a thawed stock solution was diluted in sodium bicarbonate ($NaHCO_3$) (0.6 M; pH 8.5) to provide 30 U insulin per kg body weight (BW) in 80 mL.

Glucose was dissolved in $NaHCO_3$ (0.6 M; pH 8.5) solution to give a final concentration of ~ 50%; thus providing 1 g glucose per kg BW. A combined solution of glucose and insulin was also prepared at the same relative concentrations in 80 mL of bicarbonate vehicle.

Colostrum

For each of the first 3 meals, 4 pools of colostrum were prepared as follows: primary colostrum taken up to 1 wk in advance from cows 2 to 2.5 h after calving (28.0 ± 2.29% dry matter); secondary colostrum obtained at 14 h after calving (18.2 ± 1.78% dry matter); and tertiary colostrum obtained at 26 h after calving (15.9 ± 1.44% dry matter). The pooled colostrum was stored at −18°C prior to the experiment.

Experimental procedure

One of the prepared solutions (80 mL) was administered to each calf 30 min postnatally. Group I was given insulin (30 U/kg BW); Group G received glucose (1 g/kg BW); Group IG received insulin (30 U/kg BW) and glucose (1 g/kg BW) together; while Group C received the $NaHCO_3$ (0.6 M; pH 8.5) vehicle.

Each of the 4 primary colostrum pools (1.5 L) was fed to 1 calf from each group 2 h after birth using a bucket with a teat. Similarly, 2 L of a single pool of secondary colostrum was given to 1 calf from each group 14 h after birth, while tertiary colostrum (2 L) was fed at 26 h in the same way.

Blood samples were taken by jugular venipuncture immediately before the peroral bolus (30 min postnatally), 1 h later before intake of primary colostrums, and at 32 h postnatally 6 h after the 3rd meal. Samples obtained using a sterilized needle were placed into tubes and allowed to clot spontaneously at room temperature. The serum was decanted, centrifuged at 3000 × *g*, portioned into aliquots of 1.5 mL, and stored in polypropylene microtubes at −20°C until analysis.

Laboratory methods

Concentrations of insulin, IGF-I, T3 and thyroxine (T4) in sera were measured by radioimmunoassay (RIA; INEP-Zemun, Serbia) (20,21). Intra-assay coefficients of variation (CV) ranged from 3.1% to 7.2%. For the IGF-I RIA, binding proteins were removed by acid-ethanol treatment followed by cryoprecipitation (22).

The IGFBP patterns in sera were characterized by sodium dodecyl sulphate — polyacrylamide gel electrophoresis (SDS–PAGE) and ligand-affinity blotting (23,24). Proteins were electrotransferred to nitrocellulose membrane (0.45 μm, Schleicher and Schuell), followed by autoradiography after incubation with ^{125}I IGF-I (specific activity: 36 MBq/nmol). Protein bands were putatively identified according to the mobility of reference standards: bovine serum albumin (BSA, 66 kDa), ovalbumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), and chymotrypsin (24 kDa). Autoradiographic band patterns were quantitated using the GlycoBandScan program (Version 5, 1998; PROZYME, San Leandro, California, USA). The abundance of IGFBP bands was expressed in arbitrary densitometric units (ADU).

Total serum protein concentration was determined by the biuret reaction using BSA as the standard (25). Glucose was measured spectrophotometrically using kits from Hoffman-LaRoche (Basle, Switzerland) and an automatic analyzer (Secomam CE, BP 106; Secoman, France). Assays were performed in duplicate and samples were analyzed within the same assay run.

Statistical analysis

Results were subjected to statistical analysis using the MSTATC package (Version 1.2, Michigan State University, Michigan, USA). Uniformity of variance was evaluated using Bartlett's test. One-way analysis of variance (ANOVA) was used to test for differences between

Table I. Mean serum insulin, glucose, total protein, and IGF-I concentrations in calves (n = 4) treated perorally with insulin (I), glucose (G), both (IG) or neither (C) before receiving colostrum 2 h after birth

Variable units	Insulin mU/L[a]	Glucose mmol/L	Total protein g/L	IGF-I nmol/L
Group I				
Before treatment	24.5^{B}	3.7^{D}	48.5^{BCD}	22.1^{A}
1 h after treatment (1.5 h of age)	111.7^{A}	1.5^{E}	42.3^{DE}	13.8^{AB}
32 h of age	21.3^{B}	5.0^{AB}	56.5^{ABC}	11.9^{B}
Group G				
Before treatment	23.3^{B}	3.3^{D}	47.2^{CD}	19.3^{AB}
1 h after treatment (1.5 h of age)	23.2^{B}	5.1^{ABC}	41.7^{DE}	21.9^{A}
32 h of age	20.0^{B}	5.9^{A}	57.9^{AB}	19.5^{AB}
Group IG				
Before treatment	44.1^{AB}	3.6^{D}	41.3^{DE}	18.0^{AB}
1 h after treatment (1.5 h of age)	94.0^{A}	0.9^{E}	35.8^{E}	13.0^{AB}
32 h of age	22.5^{B}	5.5^{AB}	55.1^{ABC}	22.0^{A}
Group C				
Before treatment	28.1^{B}	3.5^{D}	42.1^{DE}	18.9^{AB}
1 h after treatment (1.5 h of age)	18.8^{B}	3.8^{CD}	37.4^{E}	16.5^{AB}
32 h of age	18.4^{B}	4.2^{BCD}	59.1^{A}	13.6^{AB}
Pooled "s"	1.39	0.47	3.27	1.82
P Treatment effect	0.012	0.068	NS	NS
P Time effect	0.0065	< 0.0001	< 0.0001	0.037
P Treatment $\times$ time	0.062	0.0002	NS	0.0029

[a] Arithmetic values.

s — Standard error.

^{ABCDE} — Values in a column not sharing a superscript are significantly different ($P < 0.05$).

NS — Not significant, $P > 0.05$.

initial values for the groups of calves; non-uniformity of variance for the insulin data was corrected by logarithmic transformation (Log_{10}). Data were then subjected to repeated-measure ANOVA with 3 variables (calf, treatment, and time as a split-plot on the random treatment). When "F" for treatment, time, or interaction showed statistical significance ($P < 0.05$), differences between mean values (n = 4) were evaluated using the least significant difference (LSD) test.

Results

No differences were found between the initial mean values for the 4 groups of calves for all the variables examined. The variances were all uniform except for T4, where differences between individual animals were very large within Group G. The calves tolerated the peroral treatments well.

Serum insulin and glucose concentrations

In Groups I and IG, which were given insulin alone or combined with glucose, marked hyperinsulinemia was found 1 h later (Table I), while mean serum levels remained relatively unaltered in the other groups. These differences between the groups had disappeared at 32 h postnatally. As expected, hypoglycemia was induced in the calves treated with insulin, which the simultaneous dose of glucose did not prevent. Thus, 1 h after treatment, glucose concentrations in both groups were lower than the initial values and lower than the value for Group C at that time. Administration of glucose alone led to an increment in its mean concentration in Group G. No change was observed within the control group during the period examined.

Total protein and IGF-I concentrations

Serum total protein concentrations tended to decrease during the interval before the first feed and then markedly increased post prandially in all experimental groups (Table I). There were no significant differences between the groups.

The large F-value for interaction without a main effect for treatment, however, indicated different effects on IGF-I concentrations within the groups. Thus, while mean serum IGF-I concentrations tended to decrease in the control group and dropped significantly in Group I, the values were maintained in Group G and, after a slight dip, recovered the initial value in Group IG (Table I).

Insulin-like growth factor binding protein patterns

A typical autoradiogram for 1 calf from each group is shown in Figure 1. Generally, 4 or 5 protein bands were detected in sera. Those with Mr of 45 and 40 kDa corresponded to the well-known IGFBP-3 doublet, 34 kDa agreed with the expected mass of IGFBP-2, and 29 kDa was compatible with the size of IGFBP-1 (26,27). The 24 kDa IGFBP band compatible with the size of IGFBP-4 (26,27) was not detected consistently in all samples and was therefore not included in the analysis.

Table II. Mean serum T3 and T4 concentrations and IGFBP abundance in calves (*n* = 4) treated perorally with insulin (I), glucose (G), both (IG) or neither (C) 30 minutes after birth and before receiving colostrum at 2 h of age

Variable units	29 kDa (IGFBP–1) ADU/10 μL	34 kDa (IGFBP–2) ADU/10 μL	40 + 45kDa (IGFBP–3) ADU/10 μL	T3 nmol/L	T4 nmol/L
Group I					
Before treatment	32.3	28.5	48.2^{AB}	3.3^{D}	269
1 h after treatment	24.0	23.5	46.2^{AB}	6.3^{BC}	252
32 h of age[a]	28.6	20.2	40.9	4.8	106
Group G					
Before treatment	24.0	28.0	52.7^{AB}	3.8^{D}	257
1 h after treatment	29.2	26.1	61.0^{A}	9.4^{A}	366
32 h of age[a]	23.8	25.7	51.7	3.3	68
Group I G					
Before treatment	17.1	22.0	55.7^{AB}	4.5^{CD}	297
1 h after treatment	25.7	26.3	49.6^{AB}	6.6^{B}	304
32 h of age[a]	28.5	25.7	52.6	5.2	151
Group C					
Before treatment	23.0	24.3	47.4^{AB}	4.0^{D}	220
1 h after treatment	30.4	22.1	40.0^{B}	4.5^{CD}	232
32 h of age[a]	28.7	24.9	48.4	3.8	124
Pooled "s"	3.36	3.46	5.34	0.62	30.0
P Treatment effect	NS	NS	0.055	0.023	NS
P Time effect	NS	NS	NS	< 0.0001	NS
P treatment × time	NS	NS	NS	0.012	NS

[a] Means of values for 2 animals only; excluded from statistical analysis. ADU — arbitrary densitometric units; s — standard error; NS — not significant $P > 0.05$.

^{ABCD} — Values in a column not sharing a superscript are significantly different ($P < 0.05$).

Relative serum IGFBP-3, IGFBP-2, and IGFBP-1 concentrations

Mean values for the putative IGFBP 1-3 bands are presented in Table II in arbitrary densitometric units (ADU). Unfortunately samples at 32 h for 2 calves from each group were lost before they could be analyzed, so the results pertain to just 2 animals from each group and these have not been included in the statistical analysis. While there were large individual differences in the intensity of the bands, there were no group- or time-related differences for the putative IGFBP-1 and IGFBP-2 bands at 29 and 34 kDa. Moreover, administration of insulin did not affect the amount of IGFBP-3. One hour after peroral glucose administration, the intensity of the 40 and 45 kDa (IGFBP-3) bands was greater in Group G compared with the value for the control group.

Thyroid hormones

While there were no differences related to group or time for T4, mean T3 concentrations were observed to increase after each treatment except in the control group (Table II). The increase was greatest in the calves receiving glucose alone, leading to a higher mean value at this time than in any other group, and a treatment-time interaction for T3.

Discussion

The considerable rise in serum insulin concentrations 1 h after peroral administration alone or combined with glucose indicated that intestinal absorption of insulin had occurred in both groups (I and IG) of treated calves, leading to marked hypoglycemia. The dose and vehicle chosen were those found to be both effective and well tolerated by Pierce et al (9). Time was allowed for the effect to become apparent before the first meal of colostrum. This contrasts with the results of Grutter and Blum (10), who observed no effect after administering a 2.5-fold lower dose in saline about 4 h after birth followed immediately by a feed. Assuming a blood volume of 7% BW; however, a 100 mIU/L increment in insulin concentration represents $< 0.1\%$ of the amount applied here. The dose was > 100 times larger than the amount of insulin found in primary colostrum (28–30).

In general, Pierce et al (9) protected the insulin-treated calves by prior administration of glucose (1g per kg BW). In our case the same amount of glucose produced a favorable increase in glycemia when applied alone, but appeared to have a minimal effect on serum insulin concentrations and the induced hypoglycemia when given together with insulin. Nevertheless, at 32 h of age, mean IGF-I concentration in Group IG was similar to that in Group G and higher than in Group I. Moreover, IGF-I concentrations at 32 h in both groups that received glucose 0.5 h after birth tended to be higher than those in the control group, even though each group had consumed the same amount of colostrum in 3 meals.

Higher levels of circulating IGF-I are usually associated with increased amounts of IGFBP-3, which forms a large ternary complex unable to pass though the capillary walls into the extracellular space (31). The absence of the usual postnatal decrease in IGF-I

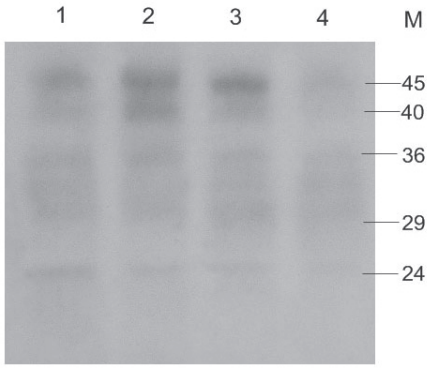

Figure 1. An autoradiogram of a ligand blot for serum IGFBPs after treatment in one calf from each group: lane 1 — insulin; lane 2 — glucose; lane 3 — insulin and glucose; lane 4 — vehicle. Positions of the molecular weight markers are indicated with dashes in line M.

concentrations in Group G was associated with higher IGFBP-3 concentrations (Table II). Hammon et al (32) showed that delaying colostrum feeding from 2 to 12 or 24 h after birth led to decreased plasma IGF-I concentrations associated with decreased IGFBP-3/IGFBP-2 ratios. In the present study, circulating IGFBP-1 and IGFBP-2 concentrations appeared to be stable and not influenced by the treatments. It is generally accepted that IGFBP-2 levels are coordinated more closely with IGF-II than IGF-I [see Delhanty and Han (33)]. Unfortunately, it was not possible to measure IGF-II concentrations in the present study, but serum levels have been reported to be about 23 nmol/L in 1-day-old calves (24) and these levels were not affected by feeding different amounts of colostrum or milk replacer or by growth hormone or long-R3-IGF-I injections (34). The lack of an effect of insulin on the putative IGFBP-1 band is interesting in the context of the well-known acute effect of insulin on its synthesis (35). Since almost all circulating IGFBP-1 is synthesized in hepatocytes, this is further evidence of the refractory nature of neonatal calf liver to the action of insulin. It seems that insulin regulates circulating IGF and IGFBP concentrations only during euglycemic clamp conditions (36,37). Moreover, insulin secretory mechanisms are not fully developed either in newborn calves or foals (10,38).

Mean thyroxine (T4) concentrations were similar to those recorded earlier for neonatal calves (2) and showed the expected time-related trend with no effect of treatment (Table II). Initial triiodothyronine concentrations were lower than those found previously (2) but rapidly increased to comparable concentrations in all except the control group. This was most pronounced in Group G, which received glucose, indicating that an early supply of available energy might favor deiodination of T4 to the more metabolically active T3. On the other hand, the hypoglycemia in groups I and IG may have decreased tissue T3 uptake, thus increasing circulating levels. Davicco et al (39) found positive linear relationships between neonatal thyroid hormone levels and birth weight in Salers and Charolais calves but not for Holstein × Friesian calves, for which the initial values were higher. Hammon and Blum (11) recorded no effect of growth hormone or long-R3-IGF-I on T3 and T4 concentrations in neonatal calves. In newborn rats, both IGFBP-2 and IGFBP-4 expression are affected by thyroid hormones independently of the somatotropic axis (18), while Ślebodiński et al (40) found that T3 and T4 had opposite effects on immunoglobulin absorption when given to neonatal calves with the first colostrum meal. The complicated interrelationship between the somatotropic and thyroid hormone axes has partially been elucidated in pigs (17). The authors concluded that the effects of thyroid status on IGF-I are mediated in part by the influence of thyroid hormones on energy balance (metabolic rate) and that nutrients can modify these effects.

Our study was initiated within the first hour after birth, unlike most other investigations which were initiated later during the first 24 h. This has confirmed the importance of a rapid supply of available energy. Namely, the group of calves that received glucose perorally 0.5 h after birth did not show a decreasing trend in IGF-I concentrations up to 32 h of age. The hypoglycemia induced by insulin administration, however, intensified the trend.

Further work is necessary to unravel the complex hormonal interactions controlling the adaptation of newborn calves to postnatal life. This will be directed at a more detailed time-related study including possible effects of prior peroral insulin and glucose treatment on serum immunoglobulin and IGF-I levels in a larger number of calves.

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